ORIGENE

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Product Information

Beta-2 Microglobulin ELISA kit

Catalog Number: EA100883
Storage Temperature: 2 – 8°C

Instruction for Use

Intended Use

The Beta 2 Microglobulin ELISA Kit is intended for the quantitative determination of Beta-2 Microglobulin (B2MG) Concentration in Human Serum.

Background

Human β-2 Microglobulin (B2MG) is an 11.8 kD protein identical to the light chain of the HLA-A, -B, and –C antigen. B2MG is expressed on nucleated cells, and is found at low levels in the serum and urine of normal individuals. B2MG concentrations are increased in inflammatory diseases, some viral diseases, renal dysfunction, and autoimmune diseases. A number of publications are available which explain the interpretation of B2MG serum levels in assessing the status of individuals with various clinical conditions.

Principle of the Test

Human β-2 Microglobulin (B2MG) is an 11.8 kD protein identical to the light chain of the HLA-A, -B, and –C antigen. B2MG is expressed on nucleated cells, and is found at low levels in the serum and urine of normal individuals. B2MG concentrations are increased in inflammatory diseases, some viral diseases, renal dysfunction, and autoimmune diseases. A number of publications are available which explain the interpretation of B2MG serum levels in assessing the status of individuals with various clinical conditions.

Components

MATERIALS PROVIDED	96 Tests
1. Microwells coated Murine monoclonal anti-B2 MG antibody	12x8x1
2. B2MG Reference Standards: 0, 0.625, 1.25, 2.5, 5, and 10	1 ml
3. Sample Diluent, 100 ml.	100 ml
4. Enzyme Conjugate Reagent, 22 ml	22 ml
5. TMB Reagent (One-Step), 11 ml	12 ml
6. Stop Solution (1N HCl), 11 ml.	11 ml
7. Wash concentrate 20X: 1 bottle	25 ml

Materials and Equipment Required but Not Provided

1. Distilled or deionized water
2. Precision pipettes

3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

Disclaimer

This product is for research use only and not intended for diagnostic procedures.

Specimen Collection Handling

1. Blood should be drawn using standard venipuncture techniques and the serum should be separated from the red blood cells as soon as practical. Avoid grossly hemolytic, lipidic or turbid samples.
2. Specimens should be capped and may be stored for up to 48 hour at 2-8°C prior to assaying. Specimens held for a longer time can be frozen at -20°C for up to 6 months prior to assay. Thawed samples should be inverted several times to mix prior to testing.
3. Collect urine samples and store at 2-8°C for up to 5 days or at -20°C for longer periods. Urine samples are diluted 1:10 by adding 50 µl urine to 450 µl sample diluent. Use same assay procedure as for serum test.

Reagent Preparation

1. **Wash Concentrate:** Prepare 1X Wash buffer by adding the Wash Concentrate (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-26°C).

Assay Procedure

- Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18-26°C). Gently mix all reagents before use
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed
- Reconstitute each lyophilized standard with 1.0 ml-distilled water. Allow the reconstituted material to stand for at least 20 minutes and mix gently. Reconstituted standards will be stable for up to 30 days when stored sealed at 2-8°C.
- It is recommended that standards, control and serum samples be run in duplicate
- Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities

Serum and Plasma

1. Samples of patient serum, plasma and control serum need to be diluted before use for best results. Prepare a series of small tubes (such as 1.5 ml microcentrifuge tubes) and mix 10 µl serum with 1.0 ml Sample Diluent (101 fold dilution). Do not dilute the standards, they have already been pre-diluted 101 fold.
2. Secure the desired number of coated wells in the holder.
3. Dispense 20 µl of standards, diluted specimens, and diluted controls into appropriate wells.
4. Dispense 200 µl of Sample Diluent into each well.
5. Thoroughly mix for 30 seconds. It is very important to mix them completely.
6. Incubate at 37°C for 30 minutes.
7. Remove the incubation mixture by flicking plate contents into a waste container.
8. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.
9. Strike the wells sharply onto absorbent paper or paper towels to remove all residual liquid droplets.
10. Dispense 200 µl of Enzyme Conjugate Reagent into each well. Gently mix for 10 seconds.
11. Incubate at 37°C for 30 minutes.

12. Remove the contents and wash the plate as described in step 7, 8, and 9.
13. Dispense 100 µl TMB Reagent into each well.
14. Gently mix for 10 seconds.
15. Incubate at room temperature in the dark for 20 minutes.
16. Stop the reaction by adding 100 µl of Stop Solution to each well.
17. Gently mix for 10 seconds. It is important to make sure that all the blue color changes to yellow color completely.
18. Read absorbance at 450nm with a microtiter well reader within 15 minutes.

Urine Test

1. Urine Samples need 10 fold Dilution with the Sample Diluent (i.e. 50 µl urine + 450 µl Sample Diluentl).
2. Follow the same Assay Procedure for Serum/Plasma Test from step 2 to step 18.

Calculation of Results

Serum and Plasma

1. Calculate the mean absorbance value (A450) for each set of reference standards, controls and patient samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in µg/ml on graph paper, with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X axis.
3. Use the mean absorbance values for each specimen to determine the corresponding concentration of B2MG in µg/ml from the standard curve.

Urine Test

1. Calculate the mean absorbance value (A450) for each reference standards, controls and patient samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in µg/ml on graph paper, with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X axis.
3. Use the mean absorbance values for each specimen to determine the corresponding concentration of β2MG in µg/ml. Divide the calculated values by 10.1 (Since the β-2 Microglobulin standards have been prediluted 101 fold, the results obtained from urine samples should be further divided by 10.1). For instance, if the calculated value for a urine sample from the standard curve is 2.40 µg/ml; then the real value will be 2.40 µg/ml ÷ 10.1 = 0.238 µg/ml.

Example of a Standard Curve

	B2MG (µg/ml)	Absorbance
Std 1	0	0.052
Std 2	0.625	0.377
Std 3	1.25	0.745
Std 4	2.5	1.414
Std 5	5.0	2.085
Std 6	10.0	2.942

Results of a typical standard run with absorbency readings at 450 nm shown in the Y axis against B2MG concentrations shown in the X axis. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Users should obtain their own data and standard curve.

Expected Values

Healthy individuals are expected to have B2MG serum or plasma values 0 - 2.0 µg/ml and urine values 0 – 0.3 µg/ml. The minimum detectable sensitivity is estimated to be 0.1 µg/ml.

References

1. Berggard I and Beam AG. 1968. Isolation and properties of a low molecular weight β2 globulin occurring in human biological fluids. J Biol Chem 243: 4095-4103.
2. Grey HM, Kubo RT, Colon SM, Poulik MD, Cresswell P, Springer T, Turner M and Stronminger JL. 1973. The small subunit of HL-A antigens is β2-microglobulin. J Exp Med 138: 1608-1612.
3. Nakamuro K, Tanigaki N and Pressman D., 1973. Multiple common properties of human β2-microglobulin and the common portion fragment derived from HL-A antigen molecules. Proc Natl Acad Sci 70: 2863-2865.
4. Evrin PE and Wibell L., 1972. The serum levels and urinary excretion of β2-microglobulin in apparently healthy subjects. Scand J Clin Lab Invest 29:69-74.
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